(12-5-91)

Accession No. 408118-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Arsenal.
Shaughnessey No. 128821.

2. **TEST MATERIAL:** AC 243,997; Lot No. 4866-62; 99.5% active ingredient; a white powder.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants.
Species Tested: Selenastrum capricornutum.

4. **CITATION:** Hughes, J.S. 1987. The Toxicity of AC 243,997 (Lot No. AC 4866-62) to Selenastrum capricornutum. Prepared by Malcolm Pirnie, Inc., White Plains, NY. Submitted by American Cyanamid Company, Princeton, NJ. EPA Accession No. 408118-02.

5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 11/29/88

Chuck Lin
12/5/91

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Isabel C. Johnson
Date: 11/30/88

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature:
Date:

6. **APPROVED BY:**

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 growth and reproduction of a non-target green alga test. With a 7-day EC50 value of 71 mg/L and NOEC value of 50.9 mg/L mean measured concentration, AC 243,997 is not expected to exert a detrimental effect on the green alga (Selenastrum capricornutum) when applied at maximum application rates up to 1.25 lbs a.i./acre.

8. **RECOMMENDATIONS:** N/A.

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9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** Selenastrum capricornutum used in this test came from laboratory stock cultures. The original culture was obtained from the National Eutrophication Research Program, U.S. EPA, Corvallis, OR. Stock cultures were maintained in a synthetic algal assay nutrient medium in Erlenmeyer flasks under constant illumination of approximately 400 foot-candles (4304 lumens/m^2) and temperature of 24 ± 2°C. Flasks were continuously shaken at 100 oscillations/min. Transfers were made regularly into fresh medium to provide 6- to 8-day old cultures for assay inoculations.

B. **Dosage:** Seven-day growth and reproduction test.

C. **Test System and Design:** Test vessels used were 250-ml sterile Erlenmeyer flasks fitted with foam stoppers to permit gas exchange. Synthetic algal assay procedure (AAP) nutrient medium was prepared with deionized water and the pH was adjusted to 7.5 ± 0.1.

Based on a range-finding test, five nominal concentrations of AC 243,997 (10, 18, 32, 56, and 100 mg/L) were selected for the definitive test. Test concentrations were prepared by adding the appropriate volumes of the stock solution (5000 mg a.i/L) to AAP medium in 250- or 500-ml volumetric flasks. After thoroughly mixing, 50 ml of each concentration were added to each of three replicate test vessels. The control contained only 50 ml medium in each of three replicate flasks. Approximately 100 ml of each test concentration and the control were retained for analysis of initial test concentrations.

The test was initiated when 0.344 ml of a 7-day-old stock culture (containing 436,000 cells/ml) was aseptically added to 50 ml of medium in each flask, yielding a nominal initial concentration of 3000 cells/ml. Flasks were kept in a Psycrotherm Controlled Environment Incubator Shaker, at a temperature of 24 ± 2°C. Temperature was recorded daily. Flasks were continuously shaken at 100 oscillations/minute and a continuous illumination of 4304 ± 650 lumens/m^2 was

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provided by overhead cool-white fluorescent lights. Flasks were randomly repositioned each working day to minimize spatial differences in the incubator.

Cell counts were made using a Coulter Counter (Model ZBI) on test days 2, 3, 4, and 7. Three counts per replicate were made. Samples were analyzed for the actual concentrations of AC 243,997 in the test solutions on day 0 and at the end of the assay (day 7).

E. Statistics: Mean cell count values at test termination on day 7 for each mean measured test concentration were expressed as a percent relative to that in the control. Percent inhibition (I) was calculated according to the following formula:

$$\% I = \frac{C - T}{C} \times 100$$

where: C = mean growth in the control,
T = mean growth in treated culture.

Note: A negative percent inhibition indicated stimulation.

To determine the EC25 and EC50 values, concentration (x-axis) was plotted against percent inhibition (y-axis). Negative percent inhibition values were included. Least squares linear regression was used to determine the line of best fit, the concentrations corresponding to 25 and 50 percent inhibition and the associated 95% confidence limits. Regression parameters were calculated using the SAS statistical package.

12. REPORTED RESULTS: The test concentrations of AC 243,997 measured on day 0 ranged from 91.4 to 100.6% of the nominal concentrations, and on day 7 from 90.4 to 109.0% of the nominal concentrations.

Table 2 (attached) presents mean cell counts during the assay. Mean cell counts were plotted against time for each test concentration in Figure 1 (attached). From the growth curves in Figure 1, the author determined that only the highest concentration of test material had inhibitory effects upon the population growth of S. capricornutum.

Effects of the test material on mean standing crop on day 7 relative to the control ranged from 5.8% stimulation to 99.9% inhibition (Table 3, attached). All test concentrations except the highest (101.2 mg/L) caused slight

stimulation of growth. The slope of the linear regression conducted on concentration versus percent inhibition was 1.1 and the correlation coefficient (r^2) was 0.79. The resulting 7-day EC25 and 7-day EC50 values were 48 and 71 mg/L, respectively. The EC25 value appears conservative given that growth stimulation was observed in the 50.9 mg/L test concentration in this assay.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusion was made by the author. Inspections had been conducted during the course of study by the Quality Assurance Unit of Malcolm Pirnie, Inc., for compliance with EPA Good Laboratory Practice Standards under the Federal Insecticide, Fungicide, and Rodenticide Act and the Toxic Substances Control Act (Fed. Reg. Vol. 48, No. 230, 11/29/83).

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

o The maximum label rate was not provided in the report. However, according to the EEB, the test substance contains 4 lbs of acid/gallon and the application rate is 2.5 pints/acre or 1.25 lbs active ingredient/acre. Therefore, if the test substance were directly applied to the surface of a 15-cm or 6-inch water column, the resulting concentration in the water would be approximately 0.92 mg/L.

o The micronutrient stock solution used to prepare the AAP nutrient medium contained 300 mg/L of $Na_2EDTA.2H_2O$. According to Subdivision J guidelines, EDTA should not be used in the experimental medium.

o The pH measurement was made in only freshly prepared medium (without test chemical). The pH should have been measured in all test solutions at test initiation and termination.

o The light intensity during the test was approximately 4.3 Klux, instead of the recommended 4.0 Klux.

o Cell counts at each treatment level were not statistically compared to the control values.

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o Observations were made only on days 2, 3, 4, and 7. Therefore, it could not be determined whether the data provided for day 7 were the maximum standing crop of the controls. Daily observations should have been taken during the test period.

B. **Statistical Analysis**: The reviewer recalculated EC50 and EC25 values using a regression analysis (attached) and obtained the same results as those calculated by the author. Analysis of variance was performed to compare cell counts at each treatment level to those of the controls (attached). The results showed that only the highest test concentration (i.e., 101.2 mg/L) reduced the cell counts of S. capricornutum at test termination (day 7).

C. **Discussion/Results**: The 7-day EC25 and EC50 values of AC 243,997 for S. capricornutum were 48 and 71 mg/L mean measured concentration, respectively. Based on the reduction of cell counts at 101.2 mg/L, the no-observed-effect concentration (NOEC) was determined to be 50.9 mg/L mean measured concentration. Therefore, AC 243,997 is not expected to exert a detrimental effect on the green alga (Selenastrum capricornutum) following normal application methods at rates up to 1.25 lbs a.i./acre.

D. **Adequacy of the Study**:

(1) **Classification**: Core.

(2) **Rationale**: Although the test procedures deviated from the guidelines, the reviewer does not believe they significantly affected the validity of the toxicity results.

(3) **Repairability**: N/A.

15. **COMPLETION OF ONE-LINER**: Yes, November 29, 1988.

PC 128821 40811802 SELENASTRUM

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Selenastrum capricornutum

DATA POINT	X	Y
1	9.4	-.6
2	16.8	-4.8
3	30.8	-.9
4	50.9	-5.8
5	101.2	99.9

REGRESSION EQUATION:
Y=-29.07039 + 1.115026 X

COEFFICIENT OF CORRELATION= .8895096

$r^2 = 0.79$

ACTUAL VERSUS ESTIMATED VALUES
X=CONCENTRATION Y=PERCENT INHIBITION

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	9.4	-.6	-18.58915	17.98915
2	16.8	-4.8	-10.33796	5.537955
3	30.8	-.9	5.272411	-6.172411
4	50.9	-5.8	27.68444	-33.48444
5	101.2	99.9	83.77025	16.12975

If Y = 50%, X = 70.91 mg/L.

∴ EC_{50} = 70.91 mg/L

If Y = 25%, X = 48.49 mg/L.

∴ EC_{25} = 48.49 mg/L

Analysis of Variance File: SELENAST Date: 01-19-1988

FILTER: None

N's, means and standard deviations based on dependent variable: COUNTS

* Indicates statistics are collapsed over this factor

Factors: C	Mean measured Conc. (mg/L)	N	Mean	S.D.
*		18	7416833.5000	3421801.5000
1	0	3	8693333.0000	323316.1600
2	9.4	3	8746667.0000	220302.8120
3	16.8	3	9106667.0000	362950.8800
4	30.8	3	8746667.0000	128582.0080
5	50.9	3	9200000.0000	277128.1200
6	101.2	3	7666.6665	3785.9390

Fmax for testing homogeneity of between subjects variances: 9190.70
Number of variances= 6 df per variance= 2.

Analysis of Variance Dependent variable: COUNTS

Source	df	SS (H)	MSS	F	P
Between Subjects	17%	19904831.3000E+07			
C (CONC)	5%	19829203.0000E+07%	3965840.7000E+07	629.263	0.0000
Subj w Groups	12%	756283340000.0000%	63023612000.0000		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	8693333.000	6	7666.667
2	8746667.000		
3	9106667.000		
4	8746667.000		
5	9200000.000		

Comparison	Scheffe'	Tukey-A*	Tukey-B*	Newman -Keuls*	Bon-ferroni	T-test	Dunnett
1 < 2							
1 < 3							
1 < 4							
1 < 5						0.0294	
1 > 6	0.0000	0.0100	0.0100	0.0100	0.0000	0.0000	0.0100
2 < 3							N.A.
2 = 4							N.A.
2 < 5						0.0472	N.A.
2 > 6	0.0000	0.0100	0.0100	0.0100	0.0000	0.0000	N.A.
3 > 4							N.A.
3 < 5							N.A.
3 > 6	0.0000	0.0100	0.0100	0.0100	0.0000	0.0000	N.A.
4 < 5						0.0472	N.A.
4 > 6	0.0000	0.0100	0.0100	0.0100	0.0000	0.0000	N.A.
5 > 6	0.0000	0.0100	0.0100	0.0100	0.0000	0.0000	N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).